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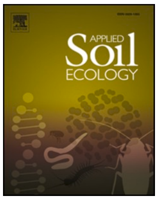

Research paper

Destabilized bacterial and fungal network weakens soil multifunctionality under increasing grazing stress

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ABSTRACT

Although it is universally acknowledged that grazing weakens most grassland ecosystem functions, the effect of varying grazing stress on soil multifunctionality (SMF) and the associated microbially-mediated mechanisms have not been fully elucidated. Here, we used a 20-year field experiment to evaluate the influence of different grazing intensities (no grazing [CK], light grazing [LG], moderate grazing [MG], and heavy grazing [HG]) on SMF and explored the regulating effect of bacterial and fungal community structure, network attributes. We evaluated 18 soil functions including soil hydrological parameters, enzymatic activities, and nutrients to characterize four individual functions (water regulation and C-, N-, P- cycling) and SMF. Our results showed that except for water regulation, SMF and other individual functions gradually decreased with increasing grazing stress. In addition, high grazing stress also reduced microbial diversity and network complexity destabilized bacterial network stability. Structural equation modeling revealed that SMF was mainly regulated by bacterial network complexity and fungal network stability. These results provide strong empirical evidence that bacterial and fungal communities have different roles in shaping SMF along grazing stresses gradient. Therefore, assessing soil multifunctionality should account not only microbial diversity but also their interactions within microbial networks. This approach is crucial for informing strategies in the degraded land restoration and sustainable utilization of grassland in arid and semi-arid ecosystems.

1. Introduction

Grasslands represent the most widely distributed ecosystem globally, covering approximately 45 % of the global land area and providing crucial biodiversity and ecosystem services for humanity (Bai and Cotrufo, 2022). Grazing, the primary use of natural grasslands, serves as a fundamental measure for ecological protection and enhancing pasture quality (Fetzel et al., 2017). While the theory suggests that moderate grazing can stimulate compensatory plant growth, boosting diversity and ecosystem functioning (McNaughton, 2022), the effect of livestock trampling and fecal-urine return on multiple soil functions (e.g. soil multifunctionality), such as nutrient cycling, and litter decomposition, remain controversial. Xu et al. (2024) found that moderate and heavy grazing can lead to a reduction in the SMF of typical grasslands. Whereas other studies have shown that grazing can substantially improve soil functions, including soil carbon (Xu et al., 2023a, 2023b), nitrogen (Yu et al., 2021), phosphorus (Yu et al., 2020) content, and belowground biomass (Cao et al., 2024). In addition, most empirical examinations of grazing effects are based on short-term grazing observations, though ecosystem responses to gazing may involve a time lag (Zhang et al., 2023b), suggesting the need for long-term experiments to investigate how livestock grazing can affect multifunctionality are needed to provide more robust evidence. Understanding the ecological mechanisms that regulating soil multifunctionality under long-term grazing is

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therefore essential to optimize grassland management and achieve sustainable grassland use.

Soil microorganisms are extremely diverse and complex, regulating almost all aspects of biogeochemical processes and playing a pivotal role in supporting SMF (Xiong and Lu, 2022). Studies suggest that biodiversity is a critical factor driving variation in individual and multiple functions in response to different environmental perturbations in grassland ecosystems (Dib et al., 2020; Mensah et al., 2020; Yuan et al., 2021b). Given the causal relationship between biodiversity and ecosystem functioning, microbial diversity can both promote SMF through complementarity and selection effects, while its loss can undermine SMF (Yu et al., 2024). Current studies report grazing decreases microbial diversity, subsequently reducing ecosystem multifunctionality (EMF) in both China (Yao et al., 2023) and typical grassland (Wang et al., 2020) However, as ecosystem responses to grazing often show a time lag, long-term experiments are essential to establish direct and reliable evidence on biodiversity-multifunctionality relationships. Bacteria and fungi are the two major microbial taxa in soil, yet differences in their survival strategies result in differential contributions to SMF (Luo et al., 2023a; Luo et al., 2023c). Moreover, although observational studies on regional (Hu et al., 2021) and global scales (Delgado-Baquerizo et al., 2016) have provided evidence for the relationship between microbial diversity and ecosystem function, disagreement about the regulatory mechanism of bacteria and fungi persists. For instance, Zheng et al. (2023) found that higher bacterial diversity, associated with functional redundancy, reduces EMF during vegetation succession in temperate grasslands, while a field manipulative experiment on Tibetan Plateau revealed that fungal community diversity dominates SMF because they are more sensitive to freeze-thaw processes (Liu et al., 2022). Thus, understanding how different microbial taxa regulate SMF in response to grazing disturbance is essential for developing sustainable management strategies. In contrast to the well-studied influence of biodiversity, the effects of microbial community composition on SMF in steppe have not been thoroughly described. Ma et al. (2022b) demonstrated that SMF across a study area within the Chinese steppe is modulated by bacteria especially *Actinobacteria* and *Proteobacteria*, but not fungi. In contrast, a transect survey suggested that fungal-dominant taxa contribute significantly more to nutrient cycling function and ecosystem stability than bacteria (Wang et al., 2022). However, these efforts have not comprehensively considered the response of bacterial and fungal compositions in the face of long-term human disturbances and their driving mechanisms for SMF. This limits the accuracy of predictions of SMF under ongoing anthropogenic influence and impedes the formulation of conservation and sustainable management policies.

A more important, yet continually overlooked indicator of microbial biodiversity is microbial interconnectedness (Faust and Raes, 2012; Fuhrman, 2009; Hallam and McCutcheon, 2015). Microbial co-occurrence networks can mechanistically reflect the systematic characteristics, overall structure, and interspecific relationships of microbial communities, greatly enriching ecological research tools (Banerjee et al., 2019; Faust and Raes, 2012; Fuhrman, 2009). In general, microbial network complexity and stability are key parameters used to characterize the degree of connectivity (Wagg et al., 2019) and the strength of interactions between microbial species (Hernandez et al., 2021), respectively. Previous work has demonstrated that microbial networks change in response to land management changes or anthropogenic disturbances (Lin et al., 2022; Xue et al., 2022). For example, grazing increases complexity within subtropical grassland bacterial networks by altering soil physicochemical properties (Ding et al., 2023). However, how microbial network properties respond to grazing intensity has not been considered in many ecosystems, including in desert steppe. Microbial networks have been shown to be critical in shaping soil function (Chen et al., 2022) and, conversely, the loss of complex associations may compromise ecosystem services and multifunctionality (Wagg et al., 2019). For example, soil erosion mediates multifunctionality by reducing network complexity in the loess and the black soil region of northwestern and northeastern China, respectively (Qiu et al., 2021). Similar findings were reported from a controlled warming experiment on the Tibetan Plateau (Chen et al., 2022). However, the driving mechanism of microbial network properties shaping SMF during long-term grazing in arid and semi-arid regions remains unclear.

The desert steppe in Inner Mongolia, with a rich grazing history, serves as both an ecological barrier in northern China and one of the most delicate vegetation types within semi-arid and arid ecosystems worldwide (Zhang et al., 2023a). Recently, the application of unsustainable management practices has accelerated soil erosion and vegetation degradation within the desert steppe, thereby exacerbating the effects of grazing disturbance (Lu et al., 2023a; Lu et al., 2023b). Therefore, a thorough understanding of the ecological consequences of this agricultural practice is necessary to maintain soil biodiversity and multifunctionality. Here, we report on a 20-year field-manipulated grazing intensity experiment to evaluate the effects of grazing on soil bacterial and fungal diversity, community composition, microbial network parameters, and soil multifunctionality. We hypothesized that (1) grazing stress reduces SMF in desert grasslands in Inner Mongolia; (2) heavy grazing reduces bacterial and fungal network complexity and stability, and that these two network attributes are coupled; and (3) bacterial diversity, community composition, network parameters drive SMF.

2. Materials and methods

2.1. Study sites and experimental design

The grazing experiment studied here is conducted in a desert steppe was in Siziwang Banner (41°46′43″N, 111°53′42″E), Inner Mongolia, China. The site is located 1456 m above s.l., the mean annual temperature and precipitation are 3.6 °C and 230 mm, respectively. The vegetation community in this study site is dominated by *Stipa breviflora, Cleistogenes songorica,* and *Artemisia frigida*.

In June 2004, a grazing platform was established on a 52.8 ha area of nature grassland with relatively flat topography and consistent vegetation and soil types. Prior to the experiment, this area was mainly used for natural grazing. Based on the theoretical carrying capacity of *Stipa breviflora* grassland as proposed in previous studies (Li et al., 2008) and a concurrent grazing density survey, 4 grazing intensities were established as follows: Control (CK, the stocking of 0 sheep/ha/half year, no sheep), light grazing (LG, 0.91 sheep/ha/half year, 4 sheep/plot), moderate grazing (MG, 1.82 sheep/ha/half year, 8 sheep/plot) and heavy grazing (HG, 2.71 sheep/ha/half year, 12 sheep/plot). The experiment was conducted in a completely randomized block design with 3 replications for each treatment, resulting in a total of 12 sample plots. Grazing took place from June to November each year, with the sheep grazing from 6 am to 6 pm daily. The sheep used in each plot were 2-year-old Ujumqin sheep, which were replaced every 3 years. If a sheep became sick during the test period, it was promptly replaced with a healthy sheep in order to ensure that the number of sheep in each plot remained fixed on an annual basis.

2.2. Soil multifunctionality

SMF was assessed according to the procedure outlined by (Maestre et al., 2012). A total of 18 soil functional indicators were used to calculate SMF, which includes C- [Soil organ carbon (SOC), Dissolved organic carbon (DOC), Microbial biomass carbon (MBC), β-D-cellobiosidase (CBH), β-1,4-glucosidase (βGC)], N- [Soil total nitrogen (STN), Soil available nitrogen (SAN), Microbe biomass nitrogen (MBN), L-leucine aminopeptidase (LAP), L-leucine aminopeptidase (NAG)], P- [Soil total phosphorus (STP), Soil total phosphorus (SAP), Microbe biomass phosphorus (MBP), alkaline phosphatase (ALP)] nutrient cycling, and water regulation [Soil bulk density (SBD), Soil saturated hydraulic conductivity (SSC), soil capillary capacity (SCC), soil capillary

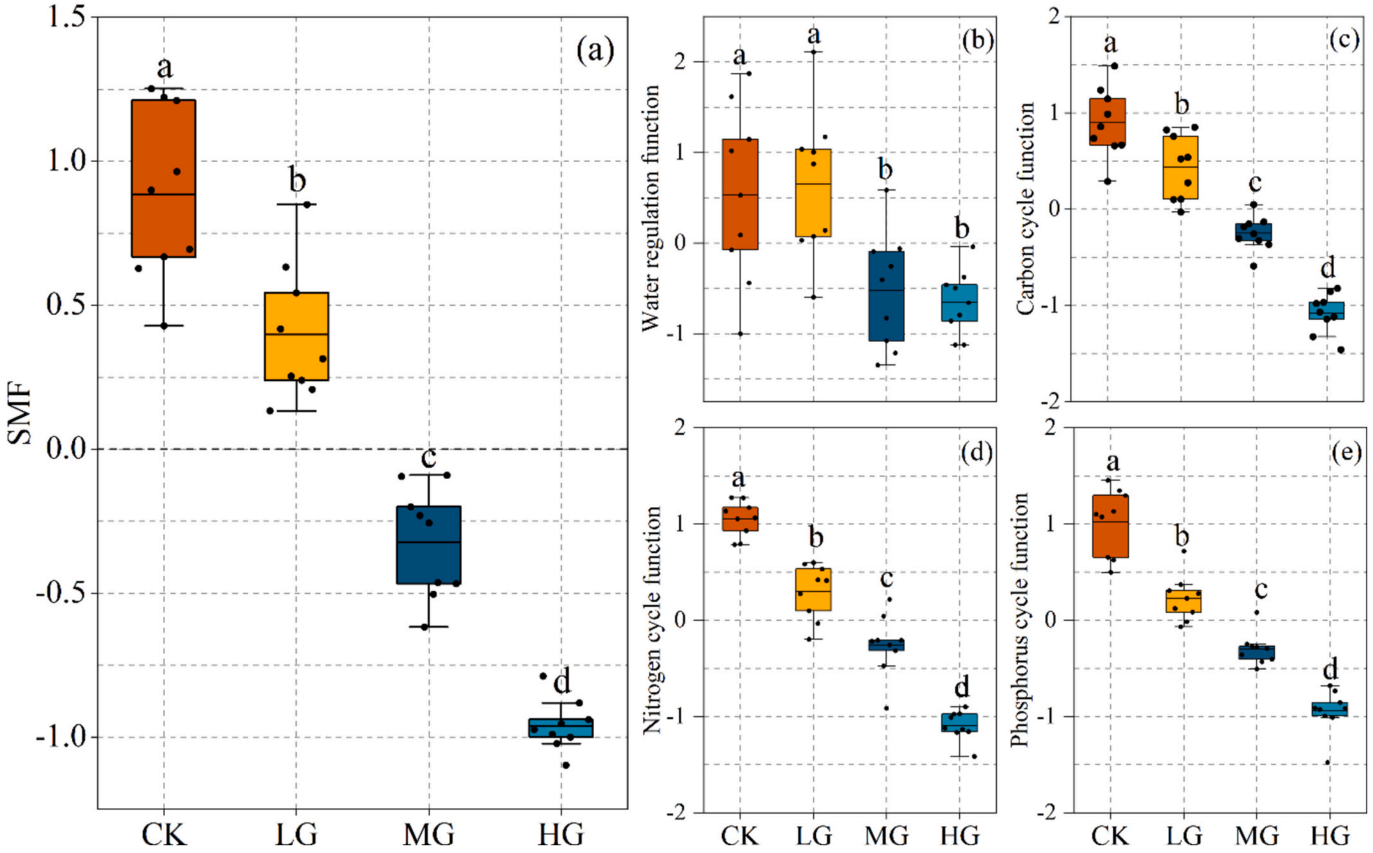

Fig. 1. The different soil multifunctionality **(a)** and individual functions related to water regulation **(b)**, carbon cycling **(c)**, nitrogen cycling **(d)**, and phosphorus cycling **(e)** along different grazing intensities (mean ± SE). Different lowercase letters indicate significant differences between means at $P < 0.05$, after using LSD tests for post hoc comparisons. CK: Non-grazed, LG: Light grazing, MG: Moderate grazing, and HG: Heavy grazing.

capacity (SFC)]. All variables were standardized using the *Z*-score method as follows:

$$Z_{ij} = (X_{ij} - u_j)/\sigma_j \quad (1)$$

where Z_{ij} and X_{ij} characterize the standardized and measured value of a soil functional variable, respectively; u_j and σ_j characterize the mean value and standard deviation of a soil function, respectively. The SMF was evaluated by averaging the transformed values of the 18 soil functions.

2.3. Soil sampling

In mid-July 2022, soil samples were collected from 3 to 10 × 10 quadrat in each grazing treatment. 5 soil cores (7.5 cm in diameter) from 0 to 10 cm surface soils were collected at 2 m intervals in each quadrant and mixed into a single composite soil sample. Stainless steel rings (100 cm^3 volume) were used to collect intact soil cores for measuring soil water regulation functions. All 36 samples were placed in sterile self-sealing bags after being sieved through a 2 mm sieve in the field to remove gravel and roots and were transported back to the laboratory in an insulated box with ice packs within 24 h. In the laboratory, soil samples were divided into three portions and placed at −80 °C, 4 °C, and room temperature for amplicon sequencing, soil enzyme, and microbial biomass measure, and soil nutrient determinations, respectively.

2.4. Amplicon sequencing and bioinformatic analyses

Using the PowerSoil DNA isolation kit (MO BIO laboratories, Carlsbad, USA), 0.25 g of soil DNA was extracted according to a standardized protocol, and each sample was repeated three times and then combined to control for error.DNA extracts were separated and purified on a 1 % agarose gel, and the concentration and purity of the extracted DNA were determined using a NanoDropUV-VIS (ND-2000c, Nano Technologies, DE, USA) spectrophotometer. Amplification of 16S rRNA and genes in distinct regions (Bac 16S: V3–V4; Fug 18S: ITS2, respectively) was conducted using specific primers (16S: 338F and 806R; 18S: ITS5-1737F and ITS2-2043R) with 12 bp barcode. PCR reactions mixture contained 25 μl 2× Premix Taq (Guangdong Magigene Biotechnology Co. Ltd., Guangzhou, China),1 μl of each primer (10 μM), and 3 μl DNA (20 ng/μl) template in a volume of 50 μl. The raw data obtained by sequencing was spliced and filtered to obtain clean data, after which operational taxonomic unit (OTU) clustering and species classification were performed. To prevent biases in bacterial and fungal diversity resulting from disparate sequencing depths, the OTU tables were subsampled to the minimum number of sequences per sample (Bacteria: 80,089; Fungi:83427 reads). Bacterial and fungal α diversity indexes (Observed OTUs, Chao1) were calculated based on rarefied OTU tables.

2.5. Network analysis

A microbial co-occurrence network was constructed from OTUs using the “Hmisc” package based on Spearman’s correlation matrices in R statistical software. Prior to network construction, the original OTUs were screened. OTUs with a relative abundance ≤1 % and a frequency of <18 occurrences in the 36 samples were removed. *P*-values for Spearman correlation coefficients and Jaccard’s dissimilarity were computed and merged using the Brown and FDR methods, and P-values were corrected by FDR. The correlation threshold was determined to be 0.65 based on the Random Matrix Theory (RMT) algorithm

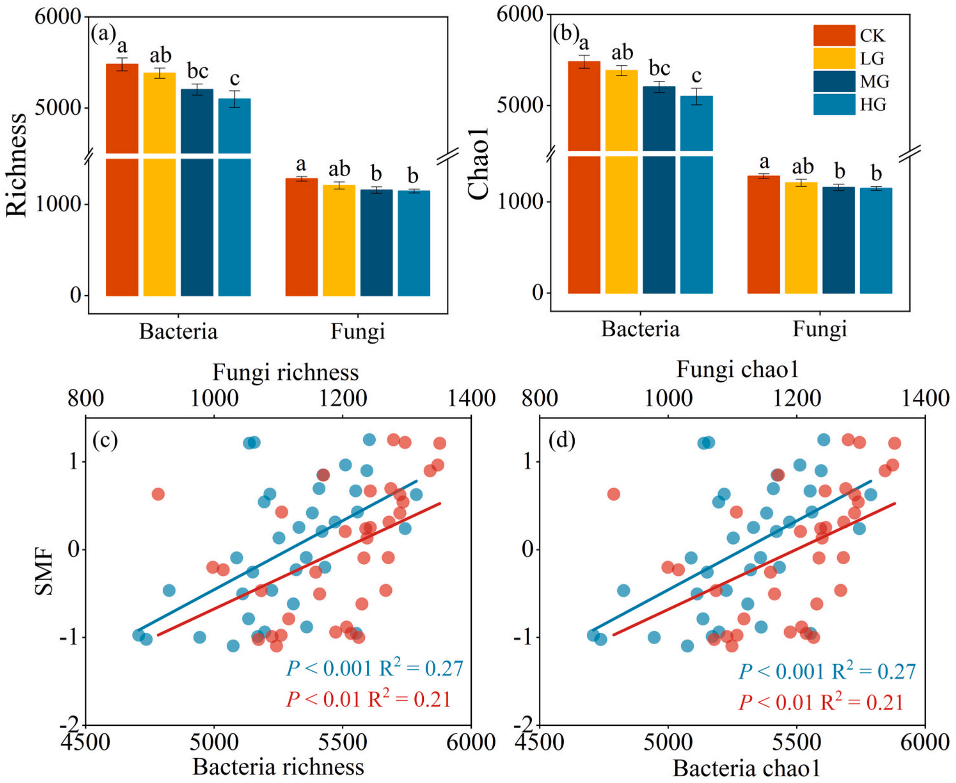

Fig. 2. Soil bacterial and fungal diversity as affected by grazing and the relationships of soil multifunctionality to diversity. Diversity of bacterial and fungal communities (Richeness **(a)** and Chao1 **(b)**) in soils from non-grazed (CK), Light grazing (LG), Moderate grazing (MG), Heavy grazing (HG), and the relationships of soil multifunctionality to diversity index **(c, d)**. Error bars are two standard errors of the mean. Means with the same lowercase were not significant at $P < 0.05$ along grazing gradients.

implemented in the RMThreshold function. Subnetwork parameters, including the number of nodes, edges, centralization, and linkage density (network complexity) were constructed and extracted for each sample by the R software with "*igraph*" package. The network images were visualized using Gephi 0.9.2. Cohesion is used to quantify microbial network stability, a connectivity matrix based on correlations between taxa. Positive and negative cohesion represents cooperative and competitive interactions between species in the community, and their ratio is defined as microbial network stability.

$$Cohesion = \sum_{i=1}^{n} r_i \times c_i \tag{2}$$

where n is the number of taxa in the community, and r_i and c_i are the abundance and connectivity of taxa, respectively.

2.6. Statistical analysis

One-way ANOVA followed by post hoc Least Significant Difference test was used to evaluate the response of SMF, soil individual function, microbial diversity, community composition, and network parameters in different grazing intensities. Relationships between microbial community diversity, network properties, and SMF were assessed using linear fitting in Origin software. To investigate the mechanisms driving SMF by bacterial and fungal communities under different grazing pressures, structural equation models (SEM) were constructed in Amos software. Notably, the first principal components (PCA1) of dominant bacterial and fungal abundance were incorporated as novel variables in SEM with the objective of evaluating the influence of community composition on SMF in R using "*corrplot*", "*factoextra*", and "*FactoMineR*" packages.

3. Results

3.1. Variation in ecosystem functioning

SMF decreased significantly with grazing intensity (Fig. 1a). Except water regulation doesn't change under light grazing (Fig. 1b), other individual functions were all affected, carbon (Fig. 1c), nitrogen (Fig. 1d), and phosphorus cycle functions (Fig. 1e) all decreasing with increasing grazing intensity.

3.2. Soil microbial diversity and community composition

Grazing resulted in marked declines in microbial diversity (Shannon and Chao1 indexes) for both bacteria and fungi (Fig. 2a, b). Diversity was significantly positively correlated with SMF (Fig. 2c, d). Dominant soil bacterial phyla were Actinobacteria (18.24–33.01 %), Acidobacteria (18.94–34.82 %), Proteobacteria (12.29–21.89 %), Bacteroidetes (5.67–14.72 %), Chloroflexi (4.57–8.27 %), Verrucomicrobia (3.89–10.27 %), and Gemmatimonadetes (4.07–8.55 %), while Planctomycetes represented the least abundant phylum (Fig. 3a). Dominant fungal phyla were Ascomycota (15.56–59.10 %), Basidiomycota (4.42–54.34 %), Mortierellomycota (0.63–23.82 %), and Chytridiomycota (0.29–16.40 %), with Glomeromycota forming the least abundant phylum (Fig. 3b). Within bacterial communities, long-term grazing resulted in a significant decrease in the relative abundance of Proteobacteria and Bacteroidetes and a significant increase in Actinobacteria (Fig. 3c). Gemmatimonadetes was the only fungal phylum affected by grazing (Fig. 3d).

3.3. Soil microbial network complexity and stability

The number of network nodes within bacterial communities did not

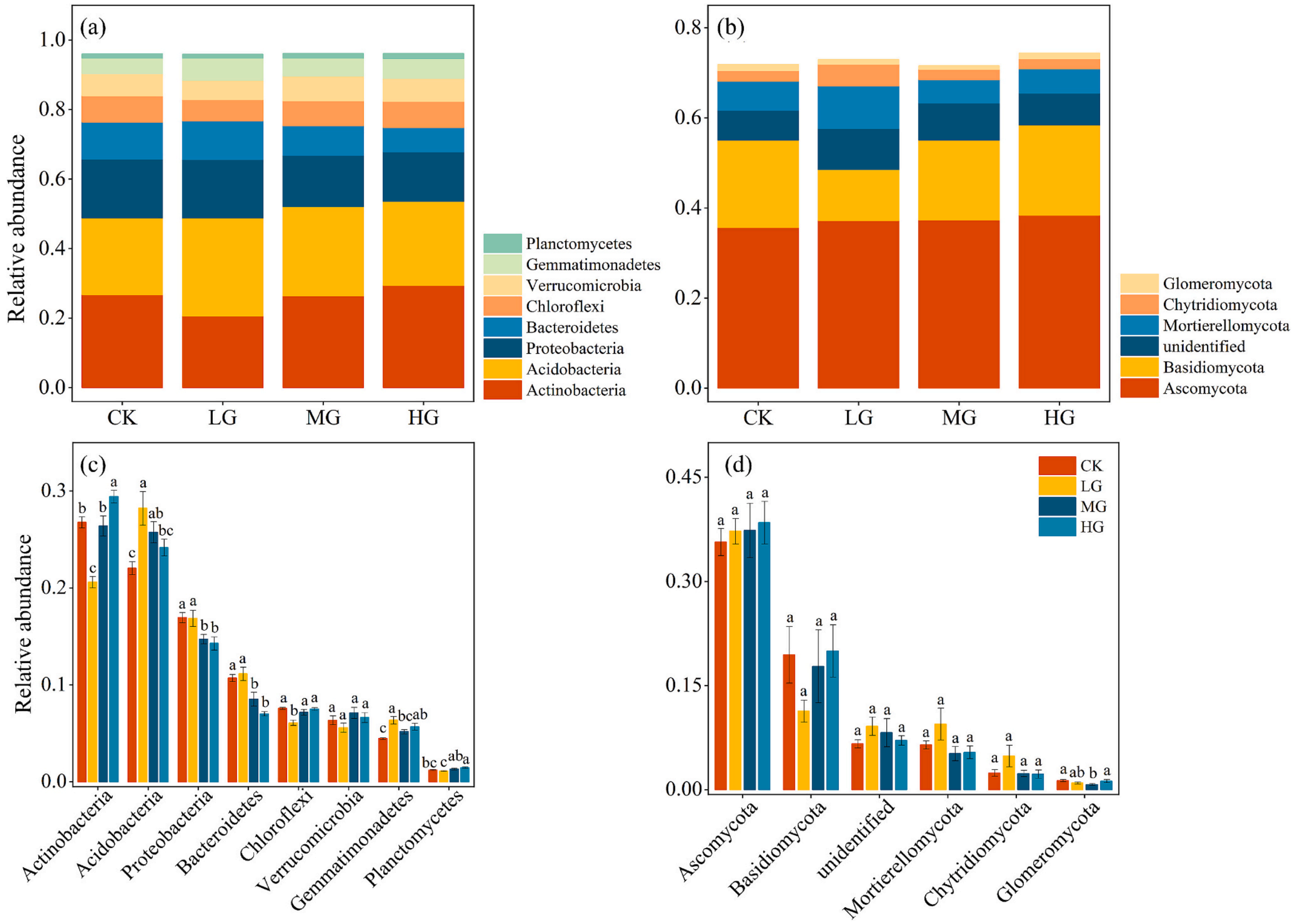

Fig. 3. The relative abundance of the dominant bacterial **(a)** and fungal **(b)** phyla. The effect of long-term grazing on the relative abundance of bacterial **(c)** and fungal **(d)** groups at the phyla level. CK: Non-grazed, LG: Light grazing, MG: Moderate grazing, and HG: Heavy grazing.

differ significantly across treatments (Fig. 4a), but HG significantly reduced the number of edges (Fig. 4b) and network complexity (Fig. 4f). In addition, betweenness centralization initially decreased before increasing with increasing grazing intensity, and LG was significantly lower than the other three treatments (Fig. 4c). The number of network nodes within fungal communities was not affected by grazing (Fig. 4a). LG maintained the edges (Fig. 4b), betweenness centralization (Fig. 4c), and network complexity (Fig. 4d) of the fungal network. We also found a positively remarkable correlation between the number of bacterial and fungal edges and network complexity and SMF (Fig. 4h). Interestingly, SMF had a significant negative correlation with the bacterial positive and negative cohesion but significantly positively correlated with stability. However, fungal positive and negative cohesion and stability were not related to SMF.

3.4. Controls on soil multifunctionality

SEM was used to further reveal mechanisms that underlie microbially-mediated changes to SMF under long-term grazing. For bacterial communities (Fig. 5a), network stability, complexity, and composition had a direct, positive influence on SMF (score at 0.93, 0.28, and 0.29), and pH had a direct, negative impact on SMF (score at −0.30). For fungal communities (Fig. 5c), network complexity and richness could explain SMF with regression weights of 0.37 and 0.34. Finally, the standardized total effect further predicted that bacterial network stability (Fig. 5b) and fungal diversity (Fig. 5d) were the main factors regulating SMF.

4. Discussion

Given that the response of individual functions and SMF to grazing, along with the underlying mechanisms in semi-arid regions, remains insufficiently explored. Our work advances current knowledge on how livestock grazing affects bacterial and fungal communities, as well as multiple soil functions, by providing long-term evidence from a 10-year field experiment. In essence, prolonged grazing has impaired the nutrient cycling and water regulation functions of desert grasslands, resulting in a consequent negative impact on SMF. Our findings reveal that long-term grazing reduces bacterial and fungal diversity and weakens the strength of their interspecific interactions (e.g., network attribute). However, we observed distinct regulatory mechanisms for SMF between bacteria and fungi, likely due to the greater sensitivity of bacteria communities to grazing disturbances. We highlight the important role of biodiversity in supporting soil functions and ecosystem services in arid and semi-arid grassland systems, emphasizing the need to consider both bacterial and fungal communities in sustainable management practices for these ecosystems.

4.1. Long-term grazing weakens individual functions and SMF

Consistent with hypothesis 1 and previous findings (Ding et al., 2023; Eldridge et al., 2016; Zhang et al., 2022), long-term grazing reduces water regulation (Fig. 1a) and nutrition cycling functions (Fig. 1b, c, d). It's widely acknowledged that gnawing and trampling by herbivores can deteriorate soil structure and reduce water and nutrient availability (Maestre et al., 2022). In addition, four individual functions

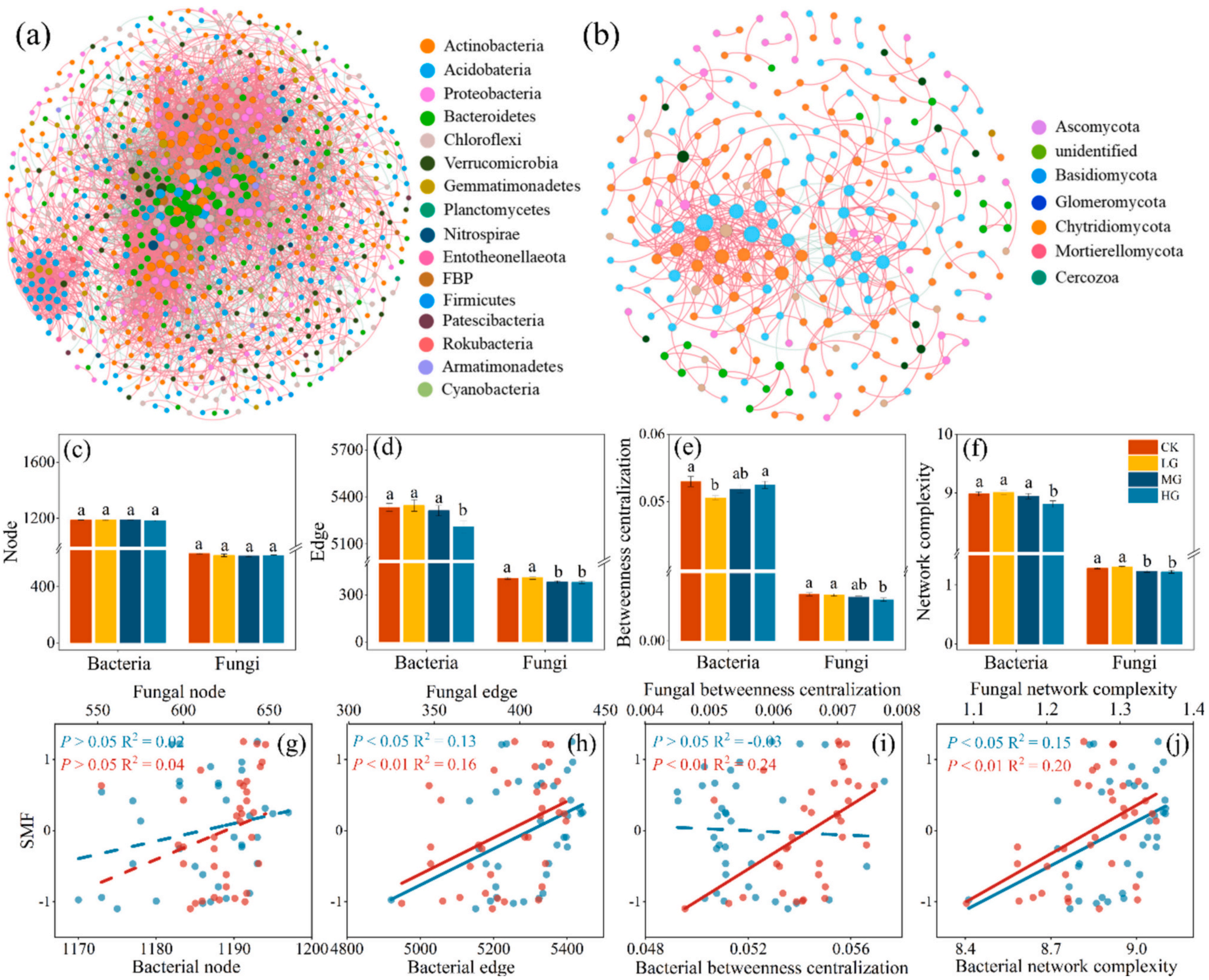

Fig. 4. Co-occurrence patterns in soil bacterial and fungal as affected by long-term grazing and the relationships of soil multifunctionality to cooccurrence assemblies. Cooccurrence network of soil bacterial **(a)** and fungal **(b)**. The numbers of node **(c)** and edge **(d)** and the betweenness centralization **(e)** and network complexity **(f)** of soil bacteria and fungi co-occurrence patterns from non-grazed (CK), Light grazing (LG), Moderate grazing (MG), Heavy grazing (HG). The relationships of soil multifunctionality to numbers of node **(g)** and edge **(h)** and the betweenness centralization **(i)** and network complexity **(j)** of soil bacteria co-occurrence pattern.

were remarkably correlated with bacterial and fungal diversity, community structure, and network properties. Grazing destroys microbial habitats, limits their metabolism, and alters bacterial and fungal community composition, thus weakening their ability to support soil function (Xun et al., 2018) Furthermore, we observed a significant decrease in SMF with increasing grazing intensity (Fig. 1a). Wang et al. (2020) reported that moderate grazing supported higher levels of biodiversity and nutrient cycling functions, which in turn enhanced SMF in Inner Mongolian grasslands. Similarly, research in Spain mountain grasslands found that free grazing can improve several soil functions such as organic matter, enzymes, and nutrients availability (Serrano et al., 2024). Inconsistent findings regarding the relationship between SMF and grazing intensity may be attributed to differences in grassland types and grazing management regimes. Additionally, contrary to our findings, a 3-year experiment showed that moderate grazing (4.80 sheep units·hm^{-2}·a^{-1}) did not affect below-ground or ecosystem multifunctionality (Wu et al., 2024), while Hao et al. (2022) discovered that short-term grazing had no notable impact on the EMF of alpine grassland ecosystems. In general, grazing effects on grassland ecosystems accumulate over time, with short-term foraging and trampling impacting plant communities first, as vegetation responds more rapidly to environmental disturbances than soil (Li et al., 2024). Therefore, evaluations of the multifunctionality must consider both grazing period as well as grassland type to improve grassland ecosystem conservation and sustainable management.

4.2. Reduced microbial diversity and community turnover along with grazing stress

Our results indicate that MG and HG reduced bacterial and fungal diversity (Fig. 2a, b). Foraging behavior by livestock may increase the loss of photosynthetic tissue and reduce below-ground C inputs via lower plant productivity driving lower soil C content (Aldezabal et al., 2015; Byrnes et al., 2018; Kawasaki et al., 2012). Because bacteria and fungi are mostly heterotrophic and use decomposing plant material as C sources to support their growth, this effect of grazing lowers bacterial and fungal diversity (Dwivedi et al., 2017; Morriën et al., 2017). Trampling by herbivores also damages soil bulk structure leading to the destruction of microbial habitats, which indirectly reduces microbial diversity. Interestingly, long-term experiments in relatively wet meadows and typical steppe of Inner Mongolia observed no significant effect of different grazing intensities on soil microbial diversity (Yu

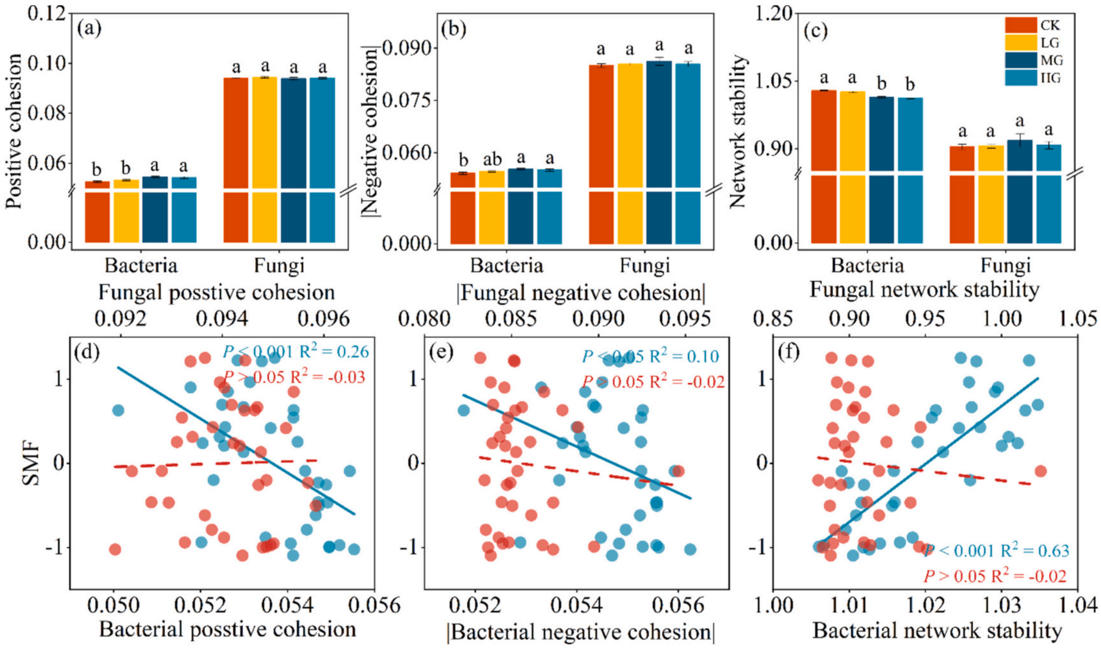

Fig. 5. Soil bacterial and fungal network stability as affected by grazing and the relationships of soil multifunctionality to network stability. Network stability of bacterial and fungal communities (Positive cohesion **(a)**, | Negative cohesion | **(b)**, and network stability **(c)**) in soils from non-grazed (CK), Light grazing (LG), Moderate grazing (MG), Heavy grazing (HG), and the relationships of soil multifunctionality to network stability index **(d, e, f)**. Error bars are two standard errors of the mean. Means with the same lowercase were not significant at $P < 0.05$ along grazing gradients.

et al., 2024). However, grazing has a remarkably reduced desert grassland bacterial and fungal diversity in our study (Fig. 2). It has been demonstrated that drought exacerbates the negative effects of grazing on microbial diversity and ecosystem functions (Maestre et al., 2015). High evapotranspiration rate and bare soil in desert steppes make them arid and fragile, and combined with the depletion of soil nutrients from long-term grazing, microorganisms cannot obtain sufficient resources from the habitat (Du et al., 2019; Zheng et al., 2021), which may reduce bacterial and fungal diversity.

Bacterial phyla exhibited specific distribution patterns with grazing intensity (Fig. 3). Actinobacteria represents one of the few groups of saprophytic microorganisms in the soil and is key to plant litter decay (Zhang et al., 2020). Several studies have reported that high nitrogen levels can promote microbial growth and thus increase the abundance of Actinobacteria (Dai et al., 2018; Zhang et al., 2018). In contrast to these findings, the HG treatment group, with the lowest N concentration in our study, had the highest abundance of Actinobacteria (Fig. 3c). Greater fecal matter deposition under HG provided a recalcitrant substrate for Actinobacteria to decompose (Zhang et al., 2020). MG and HG decrease the abundance of Proteobacteria and Bacteroidetes while promoting Planctomycetes (Fig. 3c). This result supports the microbial oligotroph-copiotroph strategy- that low nutrient availability can promote the growth of oligotrophic microbes (Planctomycetes) while reducing the coprophilous microorganisms (Proteobacteria and Bacteroidetes) abundance (Fierer et al., 2012). In addition, we observed that grazing intensity did not affect fungal community composition except Glomeromycota, consistent with the findings of (Xue et al., 2022). Because fungal communities are not as sensitive to grazing compared to bacterial communities in desert grasslands. Previous studies have confirmed that fungi exhibit generally greater stability when exposed to drought stress (de Vries et al., 2018) and other disturbances (Luo et al., 2023a).

4.3. Destabilized microbial networks with increasing grazing stress

Microbial networks represent interspecific interactions within communities and are often directly related to the intensity of environmental stresses (Hernandez et al., 2021). For instance, in northern Zhejiang province, nearshore zones with medium levels of habitat resources condition exhibited heightened microbial co-occurrence intensity, aligning with the “intermediate disturbance hypothesis” (Hou et al., 2024). Similarly, a 6-year grazing experiment revealed that moderate grazing (20–40 % utilization) increased both parasite abundance as well as microbial network complexity in mixed-grass prairies (Camuy-Vélez et al., 2024). In contrast to these findings, we found that LG did not change the soil bacterial and fungal network complexity in desert grasslands (Fig. 4). This could be due to prolonged drought and anthropogenic disturbance in desert grasslands, which may counteract their interspecific synergistic or antagonistic effects. However, with heavy grazing (HG), the resulting excessive disturbance and stronger resource constraints likely triggered ecological niche shifts, altering the strength of interspecific interactions (Freilich et al., 2018; Hernandez et al., 2021; Zhou et al., 2010; Zhou et al., 2011). Specifically, HG significantly reduced soil water content (SCC, SSC, SFC) and nutrient availability (DOC, SAN, SAP), and SCC was significantly and positively correlated with bacterial and fungal network complexity. Soil moisture is considered the main limiting factor for microbial biochemical processes and metabolic rates (Zhou et al., 2016). Thus, low water content caused by overgrazing drives strong ecological niche differentiation, and the probability of each species meeting and interacting within a short period of time may be reduced, reducing competition (Ma et al., 2016). Meanwhile, reduced soil moisture may directly constrain bacterial and fungal network complexity by hindering metabolic activity or microbial evolution and community assembly (Li et al., 2023). In addition, long-term grazing causes severe fragmentation in grasslands, forming a large number of habitat patches (Bai and Cotrufo, 2022), which reduces the number of shared ecological niches and indirectly decreases microbial network complexity (Xu et al., 2023a, 2023b). Nutrient availability modulates microbial network complexity by affecting the intensity of cooperation between soil microorganisms and the necessity of trophic interactions. This study also showed a remarkably positive correlation between microbial network complexity and DOC and SAP. Lower network complexity under HG may be partially explained by decreases in microbial biomass occurring in nutrient-deficient habitats,

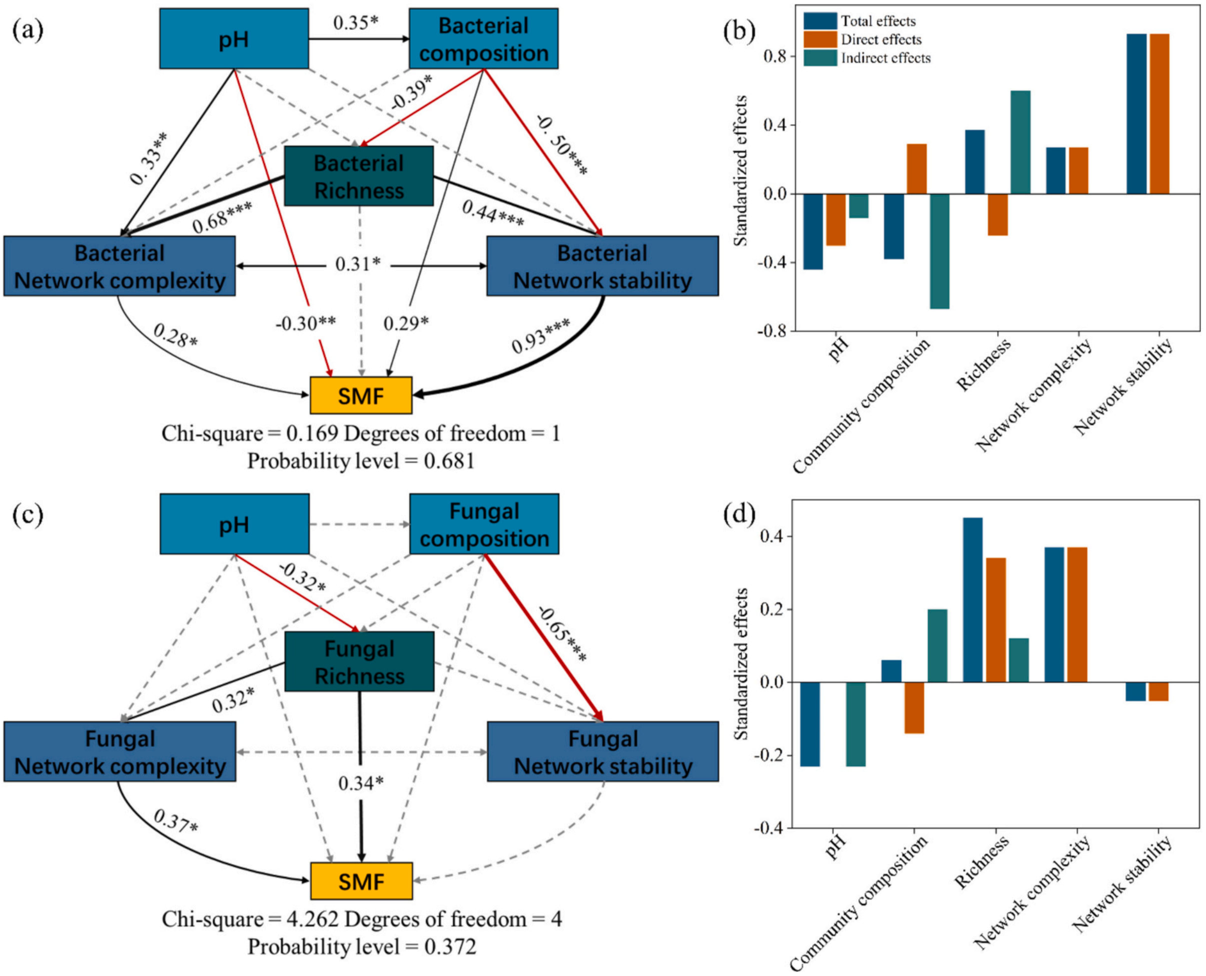

Fig. 6. Structure equation modeling (SEM) examining the standard effects of the bacterial **(a,b)** and fungal **(c,d)** community index on soil multifunctionality during long-term grazing. The black solid line, the red solid line, and its thickness represent positive and negative relationships, respectively. The Gray dotted line represents no relationships.

which opportunities for interactions between different taxa (Guo et al., 2022).

The importance of community composition especially dominant taxa, as a predictor of microbial network stability has been supported by studies in grassland (Wu et al., 2021) and cropland ecosystems (Xue et al., 2022). We observed that bacterial network stability correlated positively with coprophilic but negatively with oligotrophs. The gnawing and trampling effects of herbivores increase environmental selection pressure and tend to favor oligotrophic bacteria with the capability to improve resource-use efficiency in response to nutrient loss and to inhibit the growth of coprophilic bacteria (Wang et al., 2023b). Dependence on external resources makes commensal and oligotrophic bacteria vulnerable to environmental variability and interferes with positive interactions and co-evolution, which in turn weakens bacterial network stability (Xue et al., 2022). Interestingly, fungal network stability is not affected by grazing intensity, which may depend on their more robust community structure. Grazing did not change the composition of the vast majority of fungal communities in this study (Fig. 3d). In general, fungi are more resistant to environmental changes and perturbations than bacteria because of their physiological characteristics (i.e., active dispersal through airborne spores and aerial hyphae) (Roper et al., 2010).

Macroecology suggests that ecosystem complexity often begets stability, but the relationship between the two is poorly understood in microbial studies (Yuan et al., 2021a). We found an interesting phenomenon: network stability and complexity are significantly positively correlated with one another in bacterial communities, but they are decoupled in fungal communities. This is because bacteria and fungi respond differently to long-term grazing disturbance. Grazing significantly reduced the bacterial community complexity and stability. The slope of the fitted line was steeper for bacteria than for fungi, suggesting that bacteria were more sensitive to grazing. Previous work has shown that increased anthropogenic disturbance may select microbial networks that are more prone to sudden fluctuations and regime switches (Hernandez et al., 2021). This synergistic relationship of bacterial communities supports MacArthur's view that complexity determines stability (MacArthur, 1955).

4.4. *Driving mechanisms of SMF along with grazing stress*

The relationship between biodiversity and EMF has received much attention in recent years, especially in soil ecosystems. Soil microbial diversity contributes to the maintenance of SMF, but the relationship between SMF and soil fungal and bacterial diversity may be different (Fig. 7). SEM results showed that fungal diversity drove SMF and had the highest standardized total effect values (Fig. 6). Studies in several other ecosystems, such as forests (Hu et al., 2021), grasslands (Wang et al., 2022), and farmlands (Yang et al., 2023) found similar results. This is because fungi were central to supporting multiple soil functions compared to soil bacterial communities (Ma et al., 2022). Fungi's relatively high drought and disturbance tolerance enables them to effectively perform crucial ecological processes (de Vries et al., 2012),

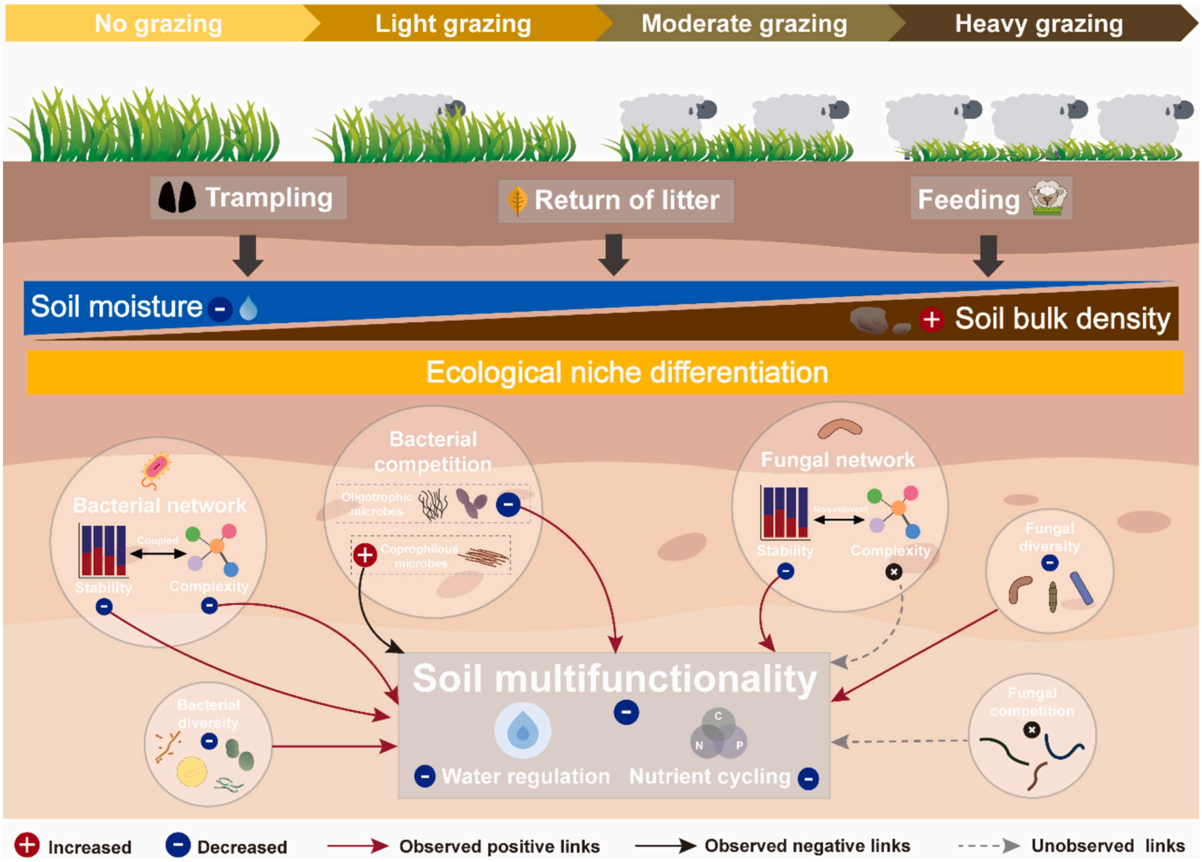

Fig. 7. Conceptual diagram for soil microbial network and soil multifunctionality during the long-term grazing.

including organic matter decomposition and cycling of C and N in semi-arid ecosystems that experience extended periods of grazing (Luo et al., 2023b). Furthermore, microbial necromass is a critical component of soil organic matter (SOM). Fungi tend to possess larger biomass; thus, fungal necromass contributes much more than bacteria to SOM stocks, supporting grassland ecosystems' function (Li et al., 2022). Our finding emphasizes that the loss of fungal diversity due to long-term grazing in desert grasslands reduces soil versatility. Therefore, we suggest that a greater emphasis should be placed on fungal communities in the conservation and enhancement of biodiversity and ecosystem services.

Consistent with our third hypothesis, bacterial community composition, not fungal, regulates SMF in desert grasslands under long-term grazing. SEM showed that bacterial community composition had a positive effect on SMF, but no significant relationship was found between fungal community composition and SMF (Fig. 6). The significance of bacterial community composition as a predictor of soil SMF is corroborated by a multitude of regional and local observations indicating that dominant bacterial taxa exert a controlling influence on SOM decomposition and formation in both natural and managed ecosystems (Bardgett et al., 2021; Delgado-Baquerizo et al., 2017; Zhang et al., 2021). We observed a significant negative correlation between Proteobacteria, Actinobacteria, and SMF, suggesting that they could mitigate reduced SMF caused by long-term grazing. Actinobacteria and Planctomycetes are considered k-strategists with oligotrophic life histories, better adapted to poor soil conditions (Delgado-Baquerizo et al., 2017). In addition, Actinobacteria and Planctomycetes were significantly correlated with enzyme activity and nutrients. In low water and nutrient habitats such as the MG and HG treatment groups, Actinobacteria and Planctomycetes participate in and support several nutrient cycling processes by releasing extracellular enzymes to break down and use recalcitrant SOM (Trivedi et al., 2013). Conversely, Proteobacteria and Bacteroidetes were significantly and positively correlated with SMF. Proteobacteria and Bacteroidetes are usually defined as r-strategists with copiotroph life histories and are more competitive in resource-rich environments (Pascault et al., 2013). However, gnawing and trampling by livestock reduces soil nutrients and inhibits key functions, such as SOM decomposition and carbon turnover (Wang et al., 2023b). In this study, Proteobacteria and Bacteroidetes were significantly and positively correlated with SOC and DOC. This provides additional support for the idea that dominant bacterial taxa are critical to SMF regulation and changes in the abundance of these taxa due to anthropogenic interference may likely alter critical soil functions.

Microbial network complexity is critical for accurate forecasts of ecosystem functions, this integration provides new research avenues for understanding microbial communities shaping ecosystem functions (Zhai et al., 2024). Partially supporting hypothesis (3), both bacterial and fungal network complexity have positive effects on SMF (Fig. 4j). Soil microbial network complexity has been confirmed in several ecosystems to be a key factor in regulating soil function (Wagg et al., 2019). Microorganisms interact to ensure nutrient acquisition and information transfer, thereby supporting multiple functions (Morriën et al., 2017). However, in desert steppe ecosystems, sparse bacterial and fungal networks under MG and HG weakened resource utilization and information transfer between microorganisms, which reduced SMF. While a growing body of research suggests that environmental changes and anthropogenic disturbances may compromise ecosystem functioning by diminishing microbial community stability (Zhang et al., 2024), there remains a notable absence of direct evidence concerning the long-term effects of grazing on the interplay between microbial network complexity and soil multifunctionality, especially in relation to various taxonomic groups of microorganisms. SEM suggests that bacterial network stability directly and positively drives SMF (Fig. 7a). Previous studies have shown that unstable network structures weaken the functions provided by ecological communities (Morriën et al., 2017; Thébault and Fontaine, 2010).

Because low-stability networks are usually accompanied by low levels of microbial competition, the impact of biodiversity on ecosystem functioning may be overestimated at this time (Wang et al., 2023a). Taken together, we found that the normalized total effect of bacterial network stability on SMF was greater than bacterial diversity and species composition, suggesting that microbially-mediated ecological processes cannot necessarily be described by the sum of their coexisting individuals (Moreno-Mateos et al., 2020). Rather, these processes are the result of integrated metabolic pathways driven by myriad interactions between individual microorganisms (Morriën et al., 2017). Microbial members with stronger connections and more frequent interactions support higher levels of ecosystem function, which may be associated with higher resource use efficiency and metabolic regulation of ecological processes (Orland et al., 2019).

5. Conclusion

A 20-year field manipulative experiment has demonstrated the response and microbial regulatory mechanisms of SMF involving nutrient cycling and water regulation in desert steppe ecosystems. Our findings indicate that long-term grazing exhibited a significant negative impact on microbial biodiversity, network complexity, and SMF. Crucially, we highlight the different roles that bacterial and fungal communities play in regulating SMF. Briefly, grazing weakens SMF primarily by destabilizing bacterial networks and diminishing fungal network complexity. These findings advance our understanding of how grazing management practices can promote soil biodiversity conservation and the sustainability of ecosystem services. Therefore, greater attention should be paid to soil microbial properties for restoring degraded grasslands experiencing long-term anthropogenic disturbance.

CRediT authorship contribution statement

Shaoyu Li: Writing – review & editing, Software, Resources, Data curation, Conceptualization. **Bin Zhang:** Writing – review & editing, Visualization, Conceptualization. **Yanan Li:** Formal analysis, Data curation. **Tianqi Zhao:** Resources, Data curation. **Jiahua Zheng:** Resources, Methodology. **Jirong Qiao:** Software, Resources. **Feng Zhang:** Visualization, Resources. **Guodong Han:** Writing – review & editing, Supervision, Funding acquisition. **Ton Bisseling:** Supervision. **Mengli Zhao:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability statement

All data generated or analysed during this study are included in this published article.

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